REMARKS

Claims 245-255, 258, 262 and 265 are pending in the above-referenced application; claims 268 and 269 have been withdrawn from consideration. New claims 270 and 271 have been added and are directed to specific embodiments. These claims are supported by the specification; no new matter has been added.

1. The Rejections Under 35 USC §112, First Paragraph (Written Description)

Claims 245, 247-255, 262 and 265 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. Several assertions were made. The various assertions made and Applicants' responses are set forth below.

1.1 Majority of insertion events inactivate a target gene

The Office Action on page 3 asserts

> ...It is agreed that the insertion of an intron into a coding sequence may inactivate gene expression. However, as set forth by applicant, 1/3 of the time, such an insertion will not inactivate gene expression. Therefore, the instant specification does not adequately describe insertion of any intron into any sequence encoding any polymerase in a manner that the construct would necessarily result in being incapable of being expressed in a prokaryotic cell and capability of producing more that one copy of a sequence, not even necessarily the same sequence, when introduced into a eukaryotic cell.

In response, Applicants disagree with the assertion that there is a lack of inadequate description of the claimed invention since 1/3 of the time an insertion would not inactivate gene expression. In Applicants view, there would actually be an expectation of success in 2/3 of the cases of a random intron sequence. Clearly undue experimentation would not be required. Furthermore, it should be understood that as noted previously, the sequences of numerous introns are known. As such, it would be a matter of routine nature to examine the sequences of a few different introns to predict which ones would result in frames shift mutations prior to carrying out any experimentation. Thus although, it would be true that "any intron" would not

necessarily introduce a frame shift mutation, it would not be difficult to identify ones that would fulfill this role.

1.2 Structural Features are recited

The Office Action on the first paragraph of page 4 recites:

> The specification does not set forth any specific structural feature to describe the genus of constructs that would result in the claimed activity. Although applicant asserts that it is a readily ascertainable property to choose an intron candidate, the specification has not described what feature of the intron would necessarily result in the instantly recited outcome. Due to the breadth of the instant claims, one of skill in the art would not be able to readily envision the instant genus of constructs that would be incapable of expression in a prokaryote but capable of producing a nucleic acid sequence in a eukaryotic cell.

In response, Applicants respectfully point out that the specification provides adequate guidance on the "specific sequence features" that are part of the intron sequences that would find use with the present invention. Thus functional features that are correlated with sequences are described. Specifically, on pages 84 and 85, it is stated that consensus sequences should be present. On page 85, it is stated that self-splicing introns and perhaps a frameshift mutation and/or stop codon should be introduced. These features would be readily discernible to the skilled user and consequently, one skilled in the art would easily be able to envision the genus of constructs that would be incapable of expression in a prokaryote but capable of producing a nucleic acid sequence in a eukaryotic cell.

In a related aspect, the Office Action on page 4 further states:

> Applicant points to the SV40 intron, as disclosed in the specification, and explains that the SV40 intron contains stop codons in all three reading frames. Applicant sets forth mathematical calculations on the probabilities of a codon being a stop codon and points to Schwartz et al. for teaching an intron inserted into a coding sequence that resulted in a frame shift mutation. Although there are introns in the art that certainly contain stop codons or would result in a frame shift mutation, the specification does not set forth any specific property that would result in incapability of being expressed in a prokaryote while able

> to produce copies of a transcript in a eukaryote and it is not evident that insertion of any intron would have these results. The Schwartz et al. reference reports on a specific intron that would result in two in-frame stop codons as well as a reading frame shift.

Applicants, in response, assert that criteria is actually set forth in the specification as to circumstances where the intron would not inactivate the gene (see page 85). Further, a reference from early studies on introns (Senapathy 1988 Proc. Nat Acad. Sci USA 85; 1129-1133, attached hereto as Appendix A and made of record in a Supplemental Information Disclosure Statement) describes the "richness" of stop codons within an intron and its possible evolutionary significance.

Applicants further assert that the incapability of expression in procaryotes or the capability of expression in eukaryotes are derived from different elements of an artificial intron insert. Specifically, the insertion of a nucleic acid segment into the coding region of a gene must convey two properties to fulfill the claim language. The first property is that the presence of this segment blocks expression. This property is derived from the internal sequences of an intron. As described above, the presence of stop codons and/or a frame shift mutation will prevent expression of a functional protein in either prokaryotes or eukaroytes. The predictability of this taking place is basic textbook Biology and would thus be clearly evident to one of ordinary skill in the art. The second property that must be conveyed, is that in a eukaryotic cell these sequences are removed, thereby also removing the blockage of expression; this property is derived from the presence of the appropriate sequences at each end of the intron as well as the flanking sequences derived from the insertion site in the coding sequence of the target of interest. Thus, in terms of each property, it is Applicants view that the teachings of the specification in combination with what is known in the art are sufficient to provide

(a) the ability to block translation of a transcript into a functional property by the presence of the stop codons and/or frameshift mutation of an appropriate intron when transcription takes place in a prokaryotic cell and

b) allow conditional expression by appropriate removal of the intron sequence when transcription takes place in a eukaryotic cell, thereby regenerating a coding

sequence that lacks the stop codons and/or frame shift derived from the insert and allowing expression of the intact protein.

1.3 The Specification does Provide Adequate Support for the Scope of the Claimed Invention

The Office Action states in the paragraph bridging pages 4 and 5 and the first paragraph of page 5 :

> It is important to note that the instant claims are not limited to the embodiments addressed by applicant above. The specification does not provide support for the use of any intron, in any polymerase or any bacteriophage polymerase, or any conditionally toxic gene, in any eukaryotic or prokaryotic cell because the specification provides only minimal description of any particular intron, polymerase (including bacteriophage polymerase), or toxic gene, or eukaryotic or prokaryotic cells for whom known structures exist that could be utilized having the claimed function.
>
> Applicant asserts that numerous polymerases were known in the art and could have theoretically been used with the present invention. However, the claims do not recite any specific structural element that would allow one of skill in the art to envision the instant genus of constructs and be able to envision which constructs would result in the instantly recited outcomes.
>
> Applicant points to Mount et al. for teachings regarding knowledge of numerous introns. It is agreed that many introns were known in the art, but one of skill would not have been able to envision which ones would act in the context of the instant claims. Regarding "toxic gene", it is acknowledged that the instant specification discusses some examples of a gene being considered toxic in a prokaryotic cell, however the instant specification does not set forth any structural feature that would allow one of skill to envision which genes are considered toxic versus those that are not within the context of the instant claim breadth.

In response, Applicants assert that as set forth in the previous response, a wide variety of introns had been sequenced by the priority date that allowed the description of the consensus sequence of splice donors and acceptors. Further,

although "any intron" may not always convey the appropriate dual properties of the expressed construct to block translation of a transcript into a functional property by the presence of the stop codons and/or frameshift mutation of an appropriate intron when transcription takes place in a prokaryotic cell and allow conditional expression by appropriate removal of the intron sequence when transcription takes place in a eukaryotic cell, there are a large number of introns that are available that will fulfill these properties and the skilled artisan is fully cognizant on how to choose such introns. Applicants further disagree with the comments made with respect to Mount on page 5 of the Office Action. It is an extremely simple matter for a user to determine the presence of stop codons as well as potential frameshifts of an intron insert from a nucleic acid sequence such that the identification of particular intron sequences that would be appropriate becomes a trivial exercise.

With respect to polymerases, the specification fully supports the use of any polymerase target where the only criterion for use is that the sequence has to be known. As stated in the previous response, numerous polymerases besides T3, T7 and SP6 were known as of the priority date of the above-referenced application. The ability of the intron to convey blocked expression in a prokaryotic cell and removal of the intron in a eukaryotic cell is not linked to the particular enzymatic function of the target. A polymerase is only recited as a specific embodiment.

Similarly, the recitation in the claims of conditional inactivation by insertion of an intron is sufficiently limited that the teachings disclosed in the specification will allow the user to practice the present invention. Specific examples are actually provided in the specification (see page 87, specifically, genes coding for toxic products, including tetanus toxin, ricin, pseudomonas toxin, *E. coli* enterotoxins, cholera toxin and other plant, animal and microbial toxins). The criteria is that the gene coding for the toxic product is maintained stably and safely in an incompatible cell and activated to produce an unaltered gene product in a compatible cell. Applicants wish to emphasize that the present invention is not conveyed by the nature of the target gene but only by the methodology of inserting an artificial intron into the target gene. No particular structural feature in the target gene is required for the practice of the invention. Thus it is understood that there is no particular

"structural feature" that would join a variety of proteins together as being toxic" but rather a functional description is being employed that would be readily understood by the user even without the benefit of the examples that were listed in the specification. Thus the joining feature is the particular action that would take place by expression of the gene.

1.4 Intron Removal is not Unpredictable

The Office Action in the first paragraph of page 6 states:

> The specification provides for the use of T3, T7 or SP6 polymerases, and also for the use of certain "consensus" splice donor and acceptor sites for inserting introns. Applicants prophetically suggest that intron "insertion at any of these sites in a gene coding region should not affect subsequent removal of the processing element in a compatible cell." (page 84 of the instant specification). However, there is significant unpredictability in such intron removal, since such a process requires a complex interaction between the nucleic acid construct and the already existent cellular machinery.

Applicants disagree. Although Applicants concede that splicing " requires a complex interaction between the nucleic acid construct and the already existent cellular machinery", the same could be said for the use of expression vectors to synthesize proteins of interest from artificial nucleic acid constructs and yet this procedure is a standard methodology of recombinant DNA. Just because complexity exists within the cell does not disallow its use, especially since the part that is manipulated by the user is only the nucleic acid and the cell provides the complex machinery that will use the nucleic acid.

1.4 The (C/A) AGG Sites

The Office Action specifically states in the second paragraph of page 6:

> Applicant argues that the *(C/A)* AGG sites in the target genes resemble a postsplice site and points to Dibb for support of this concept. Applicant argues that these sites will be converted into splice donor and acceptor sites by the addition of the flanking intron sequence. If the presence of a *(C/A)* AGG site is what applicant is relying upon for the instant mechanism to occur, this should be

> an aspect of the instant claims. Claim 262 is the only claim that requires a *(C/A)* AGG site, but the claim is directed to constructs comprising a nucleic acid sequence encoding any gene product, where the specification does not describe that this mechanism would necessarily occur in any gene with a *(C/A)* AGG site.

In response, Applicants have provided the use of "protosplice" site as an example of a preferred embodiment for carrying out the invention. By the use of a (C/A)AGG protosplice site as the insertion site, an intron can be artificially added such that the subsequent splicing event will regenerate the original (C/A)AGG) sequence afterwards, with no addition of extra amino acids added. Applicants additionally have added claims 270 and 271 to recite specific embodiments.

1.5 Balvay

The Office Action with respect to Balvay continues to assert that this reference provides evidence of the unpredictability of splicing and asserts in the paragraph bridging pages 6 and 7:

> Although applicant asserts that splicing is predictable and argues that Balvay et al. indicates that the splicing machinery is highly dependent upon recognizing and interacting with such secondary structures in making the splice and therefore demonstrates that there are additional considerations in splicing mechanisms. Balvay et al. indicates that the addition of a secondary structure to an existing mRNA can cause the cell to splice at a point not normally spliced at, while removal of such a structure can cause splicing to be eliminated (for example see pages 165 bridging to 166). Furthermore, Balvay indicates that the exon plays a significant role in splice site recognition by the cellular splicing machinery. Since one of skill would understand that the nucleotides in the exon remain in the mRNA (or ribozyme) after splicing, applicants claimed nucleic acid constructs, following splicing, would likely therefore contain elements of these exon recognition sites. Such unpredictability indicates that the genus of nucleic acid constructs comprising any intron in any polymerase (or any bacteriophage polymerase), or any toxic gene, and that are active or inactive depending on whether they are found in prokaryotic or eukaryotic cells is very large. Regardless of applicant's specific interpretation of the scenarios of Balvay et al., Balvay et

al. demonstrates that applicant has not adequately described the instant breadth in a manner that one of skill would be able to readily envision the instant constructs and would not be able to readily envision the specific genus of constructs that would result in the instant outcomes.

Applicants, in response, take issue with the reliance on Balvay. As discussed in this previous response, the Balvay references cited a number of examples where splicing took place other than expected sites, thus clearly establishing that they understood that in general these sites were predictable (and hence expected) but there were exceptions to these rules that were of interest because they were exceptions. Thus the Balvay reference describes their examples in language such as "selection of an **abnormally** located 3'splice site", "one of the **peculiarities** of this reaction", "a rather **unusual** situation" and "explains some **exceptions** to the scanning mechanisms". It is clear that the terms "abnormally', "peculiarities", "unusual" and "exceptions" are clear indication that under normal circumstances, splice sites are selected on a very predictable basis but under some circumstances violations of the standard rules take place and an explanation for these unusual and unexpected events was the gist of the Balvay reference.

Applicants also disagree with the Office Action in stating that the "the exon plays a significant role in splice site recognition" but rather conclude that when abnormal events take place the reason may reside in the presence of particular exon sequences. It also should be pointed out that the majority of the exceptions described by Balvay were not derived by oddities of the exon sequences but secondary structures present in the intron sequences. Applicants do not believe the existence of a few exceptions renders any system "unpredictable".

1.6 Lack of Disclosure of Structural Characteristic

It is continued to be asserted in the Office Action in the paragraph spanning pages 7-8 and on page 8 that there is a lack of disclosure of a structural characteristic that would envisage the skilled artisan to envisage the entire genus. Specifically, the Office Action asserts:

Furthermore, applicant asserts that the methods used to block expression are not related to the ultimate function of the protein and therefore the only knowledge necessary would be the sequence of the protein or polymerase so that an appropriate site could be chosen. However, the instant specification does not describe such a broad genus of nucleic acid constructs that would conditionally control the expression of any polymerase or protein sequence based on the presence of any intron in any eukaryotic or prokaryotic cell. The specification does not disclose a structural characteristic that would allow one of ordinary skill to recognize which introns introduced into which sequences would result in expression or lack of expression of which polymerases or proteins.

Contrary to applicant's assertions, the specific example given in the specification is not representative of the broad genus of nucleic acid constructs that are instantly being claimed. The structural characteristics recited in the instant claims are extremely broad and the specification does not disclose a structural characteristic that would allow for the skilled artisan to envisage the entire genus claimed of nucleic acid constructs with any intron that would result in any polymerase to be incapable of being expressed in any prokaryotic cells and capable of producing a nucleic acid sequence when introduced into any eukaryotic cell. Therefore, the skilled artisan would not be able to recognize that applicant was in possession of such a broad genus of nucleic acid constructs at the time of filing.

Applicant argues that one of skill is fully capable of recognizing the characteristics that would allow a user to choose a particular intron and that SV40 is an example of a wide variety of introns that would be understood to be of use in the present invention. As explained above, one of skill would not be able to readily envision the instant genus of constructs because the claims do not set forth any structural characteristic that would describe which introns inserted into sequences encoding which polymerase would result in the instant activity, as it is acknowledged in the art that there are additional considerations in splicing, as evidenced by Balvay et al., and that the breadth of the instant construct would not necessarily result in the instant outcomes.

In response to the continued assertions made in the Office Action concerning the lack of "structural characteristics", Applicants assert that structure is not required

for an adequate description of a biological macromolecule. This was articulated by the Federal Circuit in *Faulkner v, Inglis*, 448 F.3d 1357, 79 U.S.P.Q.2d 1001 (Fed Cir. 2006) which stated:

(1) examples are not necessary to support the adequacy of a written description,

(2) the written description standard may be met even where actual reduction to practice of an invention is absent, and

(3) **there is no per se rule that an adequate written description of an invention that involves a biological macromolecule must contain a recitation of known structure.**

In addition, the Federal Circuit recognizes the PTO guidelines establishing that the written description requirement can be met by showing that an invention is complete by "disclosure sufficiently detailed, relevant identifying characteristics....i.e. functional characteristics when coupled with a known or disclosed correlation between function and structure...." *Enzo Biochem Inc. v GenProbe*, 63 U.S.P.Q. 2d 1609, 1613 (Fed Cir. 2002).

With regard to the assertions at the bottom of page 8 of the Office Action stating that "the breadth of the instant construct would not necessarily result in the instant outcomes" , we believe that even if not necessarily, the constructs are <u>likely</u> to result in the instant outcomes and it would be exceptional surprises when it would not. The teachings in the specification are sufficient guidance that anyone skilled in the art should be able to practice the invention with their particular choice of intron and target with a high likelihood of success. A 100% surety is not required for an invention to be considered to be patentable.

In conclusion, Applicants assert that an adequate description of the claimed invention is provided. Thus, Applicants in view of the arguments made respectfully request that the rejection under 35 USC 112, first paragraph (written description) be withdrawn.

2. The Rejection Under 35 USC 112, First Paragraph (Enablement)

Claims 245, 247-255, 262 and 265 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The various assertions made and Applicants' response are set forth below.

2.1 Lack of Specific Examples Provided

The Office Action specifically asserts on pages 9-11:

> Applicant asserts that Example 19 provides a more than sufficient description regarding strategies used in choosing intron sequences to be used, insertion sites in the T7 polymerase and vectors, as well as construction steps. It is noted that the specific example in Example 19 is not commensurate in scope with the broadly recited characteristics of the nucleic acid constructs of the instant claims and does not reasonably provide predictability of such a broad genus of nucleic acid constructs having the instantly desired function. Although applicant asserts that there is sufficient description for choosing intron sequences, there is not sufficient description for choosing intron sequences within the context of the instant invention, as it is not evident that insertion of any intron into any sequence encoding any intron, especially wherein the insertion is at any position, would result in the instantly recited outcomes. The instant claims are not closed to introns with any specific structural characteristic, as discussed above.
>
> Applicant asserts that methods are well known in the art for introducing artificial introns. It is not disputed that methods are known in the art to introduce artificial introns. However, it is the unpredictable nature of introducing any intron into any nucleic acid sequence at any position that encodes any polymerase with a resultant incapability of the polymerase being expressed in any prokaryotic cell, whereas more than one copy of a nucleic acid sequence is produced when introduced into any eukaryotic cell. Furthermore, the claims recite that the gene product or protein expressed would be toxic specifically to a prokaryotic cell in the absence of the intron.
>
> Applicant asserts that the claims are not directed to sequences encoding any polymerase; the polymerase must be incapable of being expressed in a prokaryotic cell and capable of producing more than one copy of a sequence when introduced to a eukaryotic cell. However, the instant specification does not describe a structural

> characteristic that sets forth which polymerases are and are not within this genus. Furthermore, claims 255, 262, and 265 are not even closed to sequences encoding a polymerase but rather any gene product.
>
> Applicants' specifically claim that the inserted and inactivating intronic sequences will be spliced out, a process the specification indicates will be carried out by the cellular machinery that normally operates to splice introns out of pre-mRNA sequences. Applicants indicate that such splicing restores native activity to previously inactive proteins. However, the specification as filed does not provide any nucleic acid constructs for which this has actually been shown to demonstrate the predictability of such a broad mechanism. Applicant's specification does not provide sufficient guidance or examples that would enable a skilled artisan to make the disclosed nucleic acid constructs containing sequences that are spliced out by cellular machinery without undue experimentation. Although the specification prophetically considers and discloses making and using such constructs, such a disclosure would not be considered enabling since introducing intervening sequences into nucleic acids alters their secondary structure, which makes their ability to be cleaved by the splicing machinery unpredictable. The specification has not resolved such issues, since no exemplified constructs that contain intervening sequences and are inactive therefore, and by which later processing inside the cell restores activity. Applicants have simply not shown that such intervening sequences can be spliced out to restore any activity to previously inactive polymerases (or any toxic protein for that matter).

Applicants respectfully traverse the rejection. First, Applicants assert that the lack of a working example in itself is not an impediment and the crux of the matter seems to be an assessment of the likelihood of success. i.e the degree of predictability. Applicants further take issue with the viewpoint expressed in the Office Action that the use of an intron with known properties constitutes an unreliable element when introduced into a new environment due to speculative "secondary structure" considerations. As stated in an earlier Response, the Balvay reference may be used as post-hoc explanations of why exceptions to predicted splicing

patterns have taken place with selected nucleic acids but it is a misapplication to use it as a predictive tool.

Further, as conceded in the Office Action, methods do exist in the art for inserting artificial introns. Applicants further assert that methods are known in the art for screening whether the intron inserted would render a particular gene product incapable of being expressed in a prokaryotic cell. It is possible to experimentation would be required. However, the experimentation, in Applicants' view would not be undue.

2.2 Schwartz, Mayeda and Oshima

The relevance of Schwartz, Mayeda and Oshima continues to be questioned. Specifically, the Office Action states in the paragraph bridging pages 11 and 12:

> Applicant points to Schwartz, Mayeda and Oshima for teachings of instances where introns have been inserted and spliced in eukaryotic cells and not in prokaryotic cells. It is acknowledged that insertion of an intron into a coding sequence may result in splicing of the sequence in eukaryotic cells. However, applicant is not enabled for inserting any intron into a sequence encoding any polymerase or any gene product with a predictable effect of capability of producing more than one copy of a sequence in a eukaryotic cell while being incapable of being expressed in a prokaryotic cell. The results of Mayeda and Oshima are not enabling for a method of inserting any intron into any polymerase or gene product with the instantly recited outcomes. Mayeda and Oshima teach that determinants essential for splicing are localized in the intron itself plus 3 nt of the 5' exon rather than the overall structure of the pre-mRNA. This does not mean that the structure of the pre-mRNA is not important to the slicing process, just that the 3 nt of the 5' exon were more essential. Furthermore, Mayeda and Oshima are considered evidence that determinants/structure of the intron itself is crucial to the process, thus supporting that not necessarily any intron would result in the instant outcomes when inserted into a nucleic acid encoding any gene product or polymerase. Furthermore, the 3 nt of the 5' exon were crucial for splicing, wherein instant claim 245, for example, embraces insertion anywhere in any sequence encoding any polymerase with the instantly

> recited outcomes. Although applicant argues that intron sequences inserted into a target gene at (C/A) AGG sites are likely to be spliced out, instant claim 245, for example, does not require this. Furthermore, Balvay et al. is evidence that the target structure does in fact playa role in splicing, as discussed above.

In response, Schwartz, Mayeda and Oshima are particularly pertinent with respect to their teaching the predictability of adding introns at arbitrary sites and that surrounding exon sequences were essentially irrelevant since normal and predictable splicing reactions took place after putting the inserts into foreign environments. Although the Office Action makes the point that these references provide evidence of the importance of the intron sequences (and/or structure), it fails to make note that this then would be an intrinsic property that would be conveyed any time by a particular intron when it was used in the present invention and thus conveying a likelihood that there would be predictability of normal splice removal even in its new location regardless of the particular gene that it is inserted into. Thus, Applicants take issue with the assertion made in the Office Action that these references show that "insertion of an intron may result in splicing of the sequence in eukaryotic cells." Applicants believe that it would be more appropriate to state that in these references insertion **did** result in normal splicing and that the Balvay reference indicates that sometime it **may** result in an unexpected pattern, i.e the Schwartz as well as Mayeda /Oshima references provided concrete examples of a lack of problems while the Balvay reference (as used in the Office Action) provides only at best only a speculation of potential problems. In a certain sense it can be said that Balvay does not predict an unpredictability for insertions of introns into new locations but only provides a cautionary note of a lack of complete certainty.

Further, in response to the use of the Mayeda and Oshima references to support the requirement that claim 245 recite (C/A)AGG, Applicants note that the Mayeda and Oshima reference explicitly mentions the retention of the 5' three nucleotides as part of the insert itself, i.e. the exon sequences that make up the (C/A)AGG after a splicing event are already present in the Mayeda and Oshima insert sequences flanking the intron itself. Thus, this reference provides a specific

exemplification that sites other than a (C/A)AGG site may be used if the appropriate flanking sequences are included as well as the intron sequences themselves. Thus Mayeda and Oshima provides the basis for the conclusion that introduction of a limitation of the (C/A)AGG site is not required for claim 245.

2.3 Balvay (relevance to enablement)

Balvay is again brought up, at this point in the enablement context in the paragraph bridging pages 12 and 13. Specifically, the Office Action states:

> Applicant points to a statement of Balvay et al. "It is important to stress that in the absence of *in vivo* experiments or *in vitro* systems where transcription and splicing are coupled, all these conclusions about the functional significance of secondary structure should be taken as tentative ones." Although applicant interprets this statement as a tentative conclusion that is contrary to practical exercises that have been carried out generating *in vivo* data that introduction of introns into selected sites is a predictable art with a high likelihood of success, the statement of Balvay et al. actually supports the examiner's position. **It** is agreed that the issues of unpredictability due to secondary structure as taught by Balvay et al. could be overcome by *in vivo* experimentation, Balvay et al. is evidence that there are additional considerations such as secondary structure that would lead to unpredictability, absence evidence to the contrary. The instantly recited constructs have extremely broad structural characteristics that were not enabled by the instant specification or the state of the art at the time of filing. Although applicant asserts that Balvay is directed to special occasions, a conclusion of lack of enablement means that, the specification, at the time the application was filed, would not have taught one skilled in the art how to make and/or use the full scope of the claimed invention without undue experimentation .....

Applicants, in response, note that Balvay's disclosure contain merely post-hoc explanations of a theoretical nature. The last sentence of Balvay actually states:

> It is important to stress that in the absence of *in vivo* experiments or of *in vitro* systems where transcription and splicing are coupled, all these conclusions about the functional significance of secondary structures should be taken as tentative ones.

Balvay does not contain a single word with regard to predicting results that would take place when an intron is inserted into a new exon location and is only concerned with native RNA's that exhibit aberrant splicing patterns. Their tentative conclusions are based on a speculation of the authors trying to explain why the normal expected patterns that would normally be predicted did not take place with these exceptional cases. In Applicants view, a proper reading of this reference is that predictable patterns of splicing are the norm but aberrant examples of splicing following abnormal patterns is likely due to secondary structure considerations. As mentioned in the previous response, Applicants would even postulate that most of the Examples that are described in Balvay reference are actually now themselves predictable since they are derived from oddities of intron sequences rather than exon sequences and translocation of these particular introns into new sites would still follow the patterns they display in their normal milieu, thus making these unpredicted splicing reactions predictable .

2.4 Insertion of Introns in a Predictable Fashion

The Office Action states on pages 13 and 14:

> It is noted that introns can be inserted into genes to control the expression of the gene, as evidenced by the state of the art; (including Gatermann; and Yoshimatsu and Nagawa et al., as cited by applicant). However, none of the references are enabling for a broad method of inserting any intron into any position of any sequence encoding any gene product wherein the resultant eukaryotic sequence would express more than one copy of a sequence.
>
> Again, the issue is not whether it was known in the art how to insert introns, but rather how to insert introns in a predictable fashion in accordance with the breadth of the instant claims and have the desired outcome specific to eukaryotic and prokaryotic cells with regards to any polymerase, as recited in the instant claims. Balvay et al. is simply an example that secondary structure is one complexity when considering splicing mechanisms.
>
> In particular, it is demonstrated that the complex secondary structures of nucleic acids are responsible for their intron excision activity, and furthermore, that

> predicting the ability of the cellular splicing machinery to splice out precise intervening sequences from disrupted sequences with variable secondary structures such that native activity is restored is considered unpredictable, because the splicing machinery is sensitive to the presence or absence of such structures.

In response, Applicants assert that at the time of the application was filed the applicants had taught the use of insertion of introns into new exon sites and provided references in the original specification (Schwatrz and the Mayeda/Oshinma refernces) as well as other prior art references in previous replies (Gaterman 1989 and Yoshimatsuc 1989) where none of the problems predicted by the Examiner were observed to take place. It appears that the position taken in the Office Action that the present method is not likely to work is based upon an extrapolation of a single paper containing a purely speculative opinion. This position is a direct contradiction to published material that explicitly states that such a procedure is predictable and essentially problem free. The applicants take the position that the numerous instances where various introns are placed into new positions in various protein coding sequences with exhibition of the normal predicted splicing patterns is conclusive and definitive evidence that there is no basis of a conclusion of undue experimentation due to a "prediction" in the Office Action of unpredictable results.

2.5 Lewin

The Examiner with respect to Lewin on page 14 states

> Applicant relies on Lewin for teachings regarding experiments of splicing out a hybrid intron and teachings that splicing sites are generic, meaning that they do not have specificity for individual RNA precursors and the RNA precursors do not convey specific information (such as secondary structure) that is needed for splicing. The teachings of Lewin et al. do not diminish the unpredictability of the intron splicing mechanism when a non-native intron is inserted into a sequence having secondary structure. Simply because splice sites are generic to different sequences that do not "convey" secondary structure that is needed for splicing does not

> mean that the mechanism does not encounter problems of unpredictability as taught by Balvay et al.

In response, Applicants wish to clarify that Lewin is cited as further evidence of a general teaching of the concept concerning the conservatism of the splice signal sequences and the likelihood of a reliable and predictable series of splicing events when introns are introduced into new environments. Applicants further take issue with the assertion made in the Office action that Balvay predicts a likelihood of an unpredicted aberrant protein rather than the expected reconstituted protein derived from the required proper splicing and believe it is a misapplication of Balvay.

2.5 De Novo Determination

The Office Action on page 14 states:

> In order to practice the invention using the specification and the state of the prior art as outlined above, the quantity of experimentation required to practice the invention as claimed would therefore require the *de novo* determination of intervening sequences that can be fully spliced out without leaving behind any nucleotides that might interfere with native activity. In the absence of sufficient guidance from the specification, the amount of experimentation would be undue, and one would have been unable to practice the invention over the scope claimed.

Applicants disagree. In Applicants view, there is no need for "*de novo* determination of intervening sequences that can be fully spliced out" since the literature is replete with examples of insertion of introns that exhibit completely normal behavior when introduced into new sites.

In conclusion, Applicants assert that the enablement requirement is met. Thus, Applicants in view of the arguments made, respectfully request that the rejection under 35 USC 112, first paragraph (enablement) be withdrawn.

3. Double Patenting

Claims 245, 247-255, 262, and 265 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1

and 2 of copending application no. 11/929,055. Applicants will address this rejection once there is indication of allowable subject matter.

4. Summary and Conclusions

It is Applicants belief that the pending claims are in condition for allowance. However, if a telephone conversation would further the prosecution of the present application, Applicants' undersigned attorney request that he be contacted at the number provided below.

Respectfully submitted,

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Dated: March 15, 2009

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